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STRETCHING OF THE SKIN *IN VIVO*. A METHOD OF INFLUENCING CELL DIVISION AND MIGRATION IN THE RAT EPIDERMIS*

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Thymidine incorporated into the cell nucleus during DNA synthesis is transmitted to the progeny at the subsequent division. Tritiated thymidine may be visualized by autoradiography and has been used to investigate cell populations (1). The number of such labeled epidermal cells compared to the total basal cells is an indicator of the rate of basal cell division. For this investigation, this radioactive index is more useful than the mitotic index as the labeled daughter cells formed from a basal cell division may be observed and their migration, if any, noted (2).

The normal epidermis is a relatively slowly renewing cell population (3). Cameron and Greulich (4) pointed out that to maintain any renewing cell population, following each mitotic division an average of one daughter cell must remain in the progenitor cell pool while the other must differentiate to replace a cell which has been lost. In skin, the paired labeled cells originating from a single mitosis could either both remain in the basal layer or both ascend, or one could remain in its original location and the other migrate superficially (2). For a given division of a basal cell, any permutation may occur (5, 6).

Bullough and Lawrence (7) stated that in mouse ear epidermis most mitoses occur with the plane of division perpendicular to the basement membrane so that newly formed cells tend to remain in the basal layer. The immediate result is increased crowding and pressure within that layer. Leblond, Greulich and Pereira (8) postulated that cells are squeezed out of the basal layers by pressure exerted by an adjacent mitotic cell. To study this hypothesis we have stretched the skin in living rats to varying degrees, thereby altering basement membrane tension and the degree of crowding of basal cells, and followed the subsequent distribution of tritiated thymidine-labeled cells for 36 hours.

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MATERIALS AND METHODS

Twelve female Sprague-Dawley rats weighing 400–440 gm were kept in individual cages. In each animal, under ether anesthesia, one two cm 18 gauge stainless steel wire with pointed ends was inserted along the outer aspect of each thigh parallel to the femur. Solder aggregates were located about 3 mm from each end of one of the pins, so that when the skin of one limb was stretched to permit insertion of each end of that pin, the underlying skin would remain moderately to markedly taut as the solder aggregates prevented its return to its original length. In the other hind leg, insertion of the pin lacking the solder caused slight stretching of the underlying skin. In that limb, the skin several mm beyond the pinned area served as the true control of relaxed, unstretched epidermis. Therefore, in each rat the effects of slight, moderate and marked stretching could be compared with a control area of skin (Figs. 1–4).

Each rat was immobilized with all four limbs individually taped to pieces of tongue blades and encased in separate plaster casts. The ends of the tongue blades were secured to the cage, permitting ready access to food and water but preventing the rat from biting or scratching the casts. Tritiated thymidine (Volk Radiochemical Co., Burbank, Calif.) 200/μc/ml, was then injected intraperitoneally in a dosage of 1.0 μc/gm of body weight. A pair of rats was sacrificed by an overdose of ether at 1, 3, 6, 12, 24 and 36 hours following administration of the radioisotope. The plaster casts were then removed. In all the limbs containing the pins with the solder aggregates the skin appeared taut at the time of removal of the casts.

A block of skin and subcutaneous tissue surrounding and including the pins was excised. The block was fixed for one week in 10% neutral buffered formalin and the pin removed immediately prior to embedding in paraffin. 5 μ sections were mounted on slides coated with an aqueous solution of 0.5% gelatin and 0.05% chrome alum. They were then deparaffinized, dehydrated, washed briefly in tap water and air dried in the darkroom. Autoradiography was performed according to a slightly modified procedure of Messier and

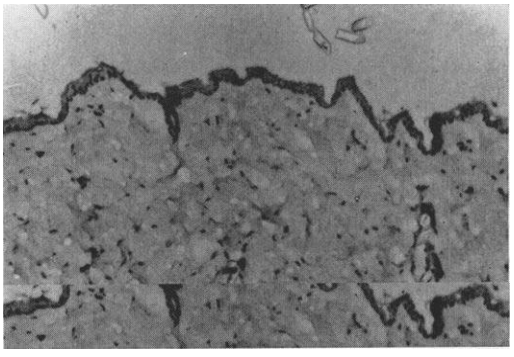

FIG. 1. Control area. Relaxed undulating skin with abundant round lymphatics. Many of the subepithelial nuclei tend to be perpendicular to the surface. (× 85).

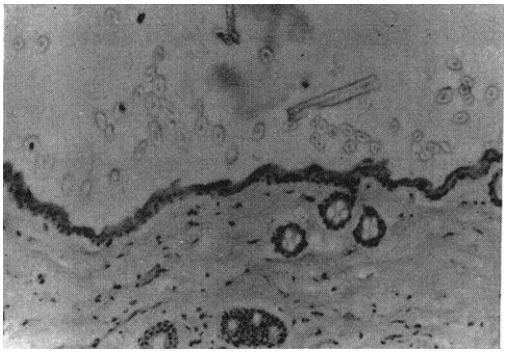

FIG. 2. Slight tension. The epithelium is thinner. Many lymphatics and subepithelial nuclei are slightly elongated parallel to the direction of stretching. (× 85).

Leblond (9). The sections were coated by dipping into Eastman Kodak NTB-2 emulsion. They were then stored in a dry atmosphere at 5°C for four weeks and developed in Eastman Kodak D-19, rinsed in distilled water and fixed for 10 minutes in acid fixer. This was followed by a 10 minute wash in running water, immersion for 2 minutes in Harris' hematoxylin and dehydration in graded alcohols. The sections were cleared in xylol and mounted. Sections were studied with their labels coded, the key being unknown to the observers. In all areas the degree of tension was estimated primarily by the amount of undulation of the basement membrane, the thickness of the epithelium and the distortion of the cells. In each section, the percentage of labeled cells, their position in the epithelium and the approximate grain count of each were recorded for the zones exhibiting differing degrees of tension. 168 sections, comprising 7 from each hind limb of each rat, were examined. The observations are recorded in Tables I and II. Those cells located in hair follicles and other cutaneous appendages were excluded as far as possible because of their higher mitotic rate (10).

The labeled spinous cells were expressed as a percentage of the basal cells from which they originated, not of the number of spinous layer cells. Comparison with the latter would not be too meaningful as almost all had arrived in that layer before the tritiated thymidine had been administered. Thus the labeled percentage would be extremely low as well as varying with the thickness of the epithelium. By counting basal cells and relating labeled spinous layer cells to them, differences in epidermal thickness could be disregarded in counting labeled cells during the 36 hour period of observation.

RESULTS

The skin was not always equally stretched throughout a section, necessitating constant appraisal of the degree of stretching as well as of the radioactivity in each area of the section.

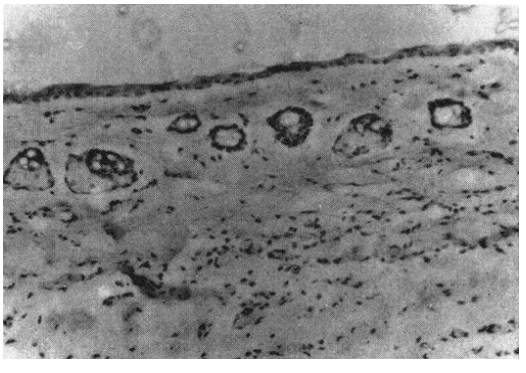

FIG. 3. Moderate tension. Thinning of the epithelium compared to previous figures. More lymphatics and subepithelial nuclei parallel the basement membrane. (× 85).

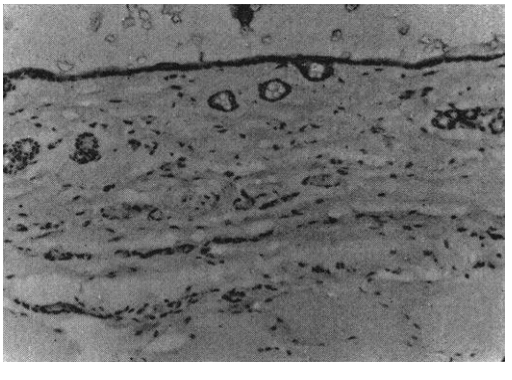

FIG. 4. Marked tension. The epithelium is very thin. Almost all lymphatics and subepithelial nuclei parallel the basement membrane. The structures in the upper portion of the figures are hairs. (× 85).

TABLE I

Percentage of labeled basal cells related to tension of rat epidermis

Hours after ^{3}H-T*	Rat #	No additional tension	Slight tension	Moderate tension	Marked tension
		%	%	%	%
1	1	0	0.11	0.32	0
	2	0.21	0.37	0.11	0
3	3	0.16	0.45	0.30	0
	4	0.24	1.06	0.87	0
6	5	1.00	1.64	0.82	0
	6	1.15	1.63	0.93	0
12	7	1.56	1.86	1.35	0
	8	1.25	1.75	1.19	0
24	9	2.10	2.54	1.41	0
	10	1.86	2.60	0.98	0
36	11	4.24	6.19	2.21	—
	12	0.80	1.75	0.69	—

* tritiated thymidine (1 μc/gm) administered intraperitoneally. Seven sections of each hind limb of each rat were examined.

The percentages of labeled compared to unlabeled cells in Tables I and II should be regarded as careful estimates rather than precise values for the following reasons: 1) The number of labeled cells in different sections from a given limb varied, reflecting the randomness of mitosis and migration (5). 2) The relationship between the plane of sectioning and the epidermis was not always constant (11). 3) A given section may not have passed through the nuclei of all the cells. 4) The numerous cutaneous appendages had more mitoses than the skin itself. Despite attempts to avoid them in counting, undoubtedly some of the labeled cells represented hair cells rather than those of the intervening epithelium. 5) Stretching caused cells that would otherwise obviously be in the spinous layer to approach the basal layer so they might, at times, be erroneously counted as basal cells. 6) Markedly stretched cells were so thin and dense that were tritiated thymidine present it would have been very difficult to see the black grains overlying their dense nuclei.

At the puncture sites hemorrhages occurred. In the later specimens microabscesses often formed. The areas near these sites were not included in the data.

In areas of skin without additional stretching there was a rapid and then a more gradual increase in basal cell labeling after tritiated thymidine (Table I). The early values may reflect not only cells entering the DNA synthetic phase (Fig. 5) but progressive absorption from the peritoneal cavity making more isotope available for labeling. At 12 hours and beyond, the increased percentages largely reflect the division of previously labeled cells into daughter cells which usually remained in the basal layer, even at 36 hours. Slight stretching increased the labeling of basal cells (Table I, Figs. 5, 6). Further tension either increased labeling somewhat or tended to reduce it (Tables I and

TABLE II

Ratios of labeled spinous layer cells to the total number of basal cells expressed as percentages

Values for different degrees of stretching are tabulated

Hours after ^{3}H-T*	Rat #	No additional tension	Slight tension	Moderate tension	Marked tension
		%	%	%	%
1	1	0	0	0	0
	2	0	0	0	0
3	3	0	0	0	0
	4	0	0	0	0
6	5	0	0	0	0
	6	0	0	0	0
12	7	0.04	0.04	0	0
	8	0	0	0	0
24	9	0.40	0.06	0.18	0
	10	0.20	0.20	0.07	0
36	11	2.42	1.52	0.24	—
	12	0.24	0.17	0.11	—

* see footnote Table I.

FIG. 5. Control area. 3 hours after tritiated thymidine (^{3}H-T). Relaxed, undulating skin. One labeled basal cell is present. Background grains of isotope are visible throughout the figure. (× 340).

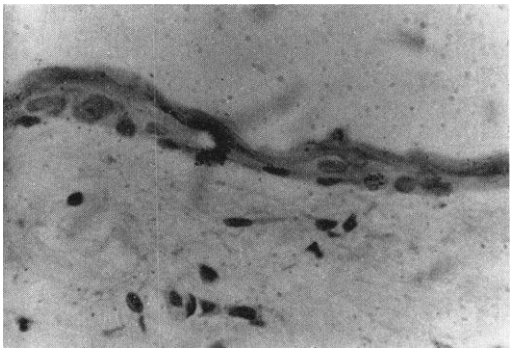

FIG. 6. Moderate tension. 3 hours after ^{3}H-T. Two labeled basal cells are present. (× 340).

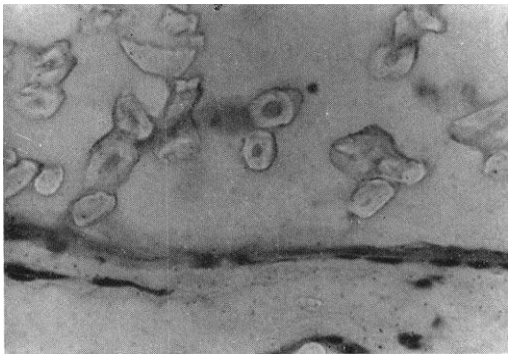

FIG. 7. Marked tension. 3 hours after ^{3}H-T. No labeled cells are seen in the very thin epithelium. Note the collapse of the subepithelial lymphatics. (× 340).

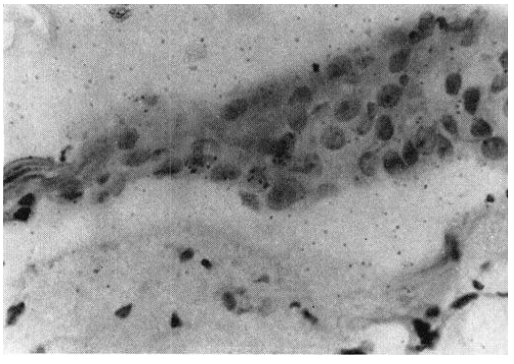

FIG. 8. Control area. 36 hours after ^{3}H-T. Two labeled basal cells are present. Above the one on the right a labeled daughter cell is seen in the spinous layer. (× 340).

II). The decreased number of labeled basal cells was unaccompanied by a corresponding increase of labeled spinous cells. Thus the diminution in the former was not due to migration.

In both the control and stretched areas, the spinous layer cells were labeled later and in a much lesser percentage than the basal cells, particularly during the first 24 hours. Any degree of tension usually inhibited cell ascension, marked stretching preventing it entirely (Table II, Fig. 7).

Unstretched rat epidermis has an undulating basement membrane with columnar basal cells. Even slight tension deformed the basal cells so that they were no longer columnar but cuboidal. Greater stretching caused them to become thinner and elongated parallel to the basement membrane. They stained densely with hematoxylin in contrast to corresponding cells in unstretched areas. Not only did individual epithelial cells elongate but the distance between them increased with progressive

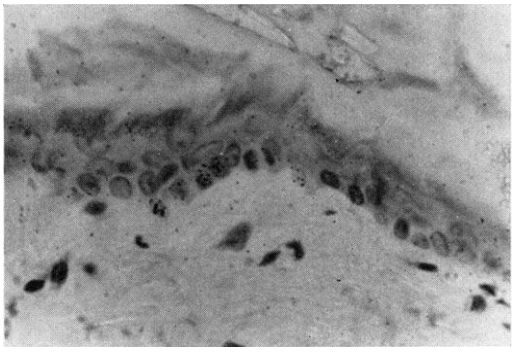

FIG. 9. Slight tension. 36 hours after ^{3}H-T. Four labeled basal cells are present. The center two are daughter cells, both of which are on the basement membrane in contrast to the preceding figure. The epithelium is thinner than in Fig. 8. (× 340).

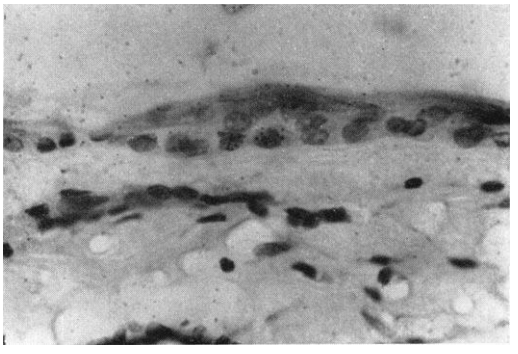

FIG. 10. Moderate tension. 36 hours after ^{3}H-T. Two adjacent basal cells are daughter cells. The nuclei are more separated and the epithelium is thinner than in the preceding figure. (× 340).

tension (Figs. 5–11). Stretching altered the spinous and other layers similarly. With progressive stretching the epithelium became thinner as did the subepithelial cell nuclei and lymphatics (Figs. 1–4). All tended to become oriented parallel to the axis of stretching.

The first daughter cells formed from basal cell division were seen in the control areas twelve hours after isotope administration. Their number increased thereafter. In the control areas, either both were in the basal layer or one lay adjacent in the spinous layer. The application of even slight tension typically caused both daughter cells to rest on the basement membrane (Fig. 9). Nonetheless, even with moderate stretching at 36 hours some daughter cells entered the spinous layer (Table II).

The grain count of the labeled basal cells at 36 hours was diminished compared to those seen earlier indicating that most represented daughter cells. The latter have half the radioactivity of their immediate progenitors (2). The additional cells formed from the mitoses, together with possible alterations in the collagen of the dermis, would have diminished skin tension and may have accounted for the almost constant failure to observe marked stretching 36 hours after the injection of tritiated thymidine (Fig. 11, Tables I and II).

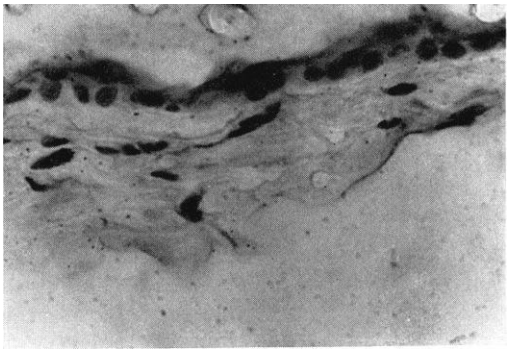

FIG. 11. Increasing tension. 36 hours after ^{3}H-T. No definite basal cell labeling. It was not possible to achieve marked tension at that time. The epithelium is thinner than in the previous figures and few patent lymphatics or capillaries are present near the basement membrane. (× 340).

DISCUSSION

Our findings for unstretched skin are, in general, similar to those of others in that two hours after the injection of tritiated thymidine 0.4% of the basal cells contained the isotope (12), that by eight hours 2% of the nuclei were labeled (1) and that by 12 hours adjacent labeled daughter cells were often seen (2). Our data are also compatible with those of Fukuyama and Bernstein (13) who report that few tritium-containing spinous layer cells appear until 12 hours after injection, but by 36–48 hours their number is greatly increased.

A number of interrelated factors may explain the increased basal cell labeling which usually accompanied slight or moderate stretching of the skin (Table I). 1) Tensing the epidermis from its surface might have made the superficial layers more resistant to upward migration from the deeper ones. 2) Tension separated the cells, perhaps facilitating the daughter cells remaining in their germinative layer. 3) Elongation of the basal cells expanded their surface area in contact with the basement membrane. Thus, unless the circulation were concomitantly reduced, more tritiated thymidine and nutrients may have been absorbed by each cell promoting labeling and mitosis. 4) If slight venous stasis were induced by stretching, the isotope might have remained in the vicinity of the cells unusually long, likewise favoring increased labeling.

Increased mitoses may well have been a means of restoring the shape of the cells from the alterations induced by stretching. Cell contact is said to inhibit cell division, perhaps by reducing the area to which nutrients have access (14), so that stretching, as massage (15), by decreasing cell contact may promote mitosis. Lessened deformation of individual basal cells would result as their number increases. This may be a possible feedback mechanism for restoring the shape and intracellular tension of the basal cell population. When this eventually occurs, it would be expected that the remaining layers would then be reconstituted.

Possibilities to explain the reduction and absence of labeling in moderate or extreme stretching are that the induced cell deformity may have severely reduced the cells' reproductive capacity or the collapse of the fragile vessels decreased the opportunity for nutrients or tritiated thymidine to reach the cells. Differences in vascularity, such as presumably have been induced by stretching, may affect the intermitotic time interval (16). Kenedi *et*

al. (17) said that tensing skin may close the small blood vessels and damage tissue. Pressure bandages also decrease both the blood supply and cell replacement (18).

Stretching might also affect cell division by influencing possible mitotic stimulators (19), or inhibitors termed chalones (20). However, these have not been investigated. Neither has the possibility that stretching might affect the innervation of the skin. Silen *et al.* showed that vagotomy increased the rate of proliferation of intestinal epithelium (21).

Ryan (18) observed that in old age the rete pegs become flat. The number of mitoses likewise increase (22). These alterations resemble the present observations in the slightly stretched skin of young adult rats.

In vitro studies have demonstrated that mechanical strains in skin may be absorbed by the elastic alpha-keratin filaments of the epidermal tonofibrils (23) accompanied by straightening and orientation rotation of the collagen bundles (24) with extrusion of tissue fluid (25).

Although no previous *in vivo* observations on the effect of stretching on cell division and migration have been made, several *in vivo* studies are of interest. Pinkus (26) noted that when the epidermis is stretched in inflammation and edema there are fewer cells in a given length of tissue. Brophy and Lobitz (27) stretched the skin of the back in man before applying cellophane tape to study mitoses in stripped epidermis. The present data suggest that the stretching may have unknowingly affected their findings, as well as of those who investigated the uptake of tritiated thymidine following intradermal injection into normal (28, 29) and psoriatic (29) human skin. An intradermal injection balloons the skin until the fluid is absorbed. Therefore, data obtained shortly after such a procedure may not be truly representative of normal or diseased skin.

Alterations in many tissues secondary to modifications of tone are frequent. The urinary bladder, uterus, arterioles and heart become hyperplastic in response to increased pressure in their luminal surfaces. Bone, muscle and connective tissue also respond structurally to changes in tension (30). It should not be surprising that the epidermis behaves similarly. Changes in volume and presumably tension occur in many organs and systems, i.e. cardiorespiratory, gastrointestinal, urinary bladder and the uterus. Could these alterations affect their rates and patterns of cell division?

Stretching of the skin is not merely an experimental procedure but is a very common clinical occurrence. Stretching of the abdominal skin occurs in pregnancy, ascites and Cushing's syndrome. Stretching of the skin of the breasts occurs in pregnancy and lactation. In the extremities it occurs normally as joints move, and pathologically when edema accumulates. It also may occur following implantation of silicones or other materials to enlarge or support the tissues of the breast or face. In addition, skin or mucous membrane overlying a tumor or abscess may be stretched and transient stretching occurs following parenteral injections. Conversely, skin tension decreases in cachexia and lipodystrophy and after rapid weight loss, childbirth, paracentesis or diuresis.

Stretching of the skin is important in plastic surgery. Gibson and Kenedi (31) point out that with time stretched skin will relax as the skin "creeps." This may be due in part to increased epidermal mitoses secondary to the increased tension. This mechanism might be operative in the normal growth process or in acromegaly. It is possible that if cartilage or bone enlarge before the surrounding soft tissues do, slightly increased soft tissue tension in the direction of growth would occur, thereby stimulating cell division. The skin and other tissues would then enlarge secondary to the growth of the skeletal tissues until the increased tension is relieved.

In areas of extreme tension essentially no mitoses occur. Thus, the skin would become thin not only because of the stretching but because the mitotic rate could not thereafter compensate for normal desquamation or injury. This might predispose to ulceration in edematous areas and likewise help explain the "pointing" of abscesses.

SUMMARY

To investigate whether crowding in the basal layer of the skin affects cell division and migration, the skin in living rats has been stretched to varying degrees by the insertion of stainless steel pins. By autoradiography, the subsequent percentage and distribution of tritiated thymidine-labeled epidermal cells have

been followed for 36 hours in the stretched and in control areas.

Progressive tension elongates and flattens epidermal cells and separates adjacent cells, accompanied by narrowing of the subepithelial vessels. Slight stretching at first increases the labeling of basal cells compared to control areas. With further tension a point is reached when the labeling percentage decreases. Marked tension consistently prevents labeling. Any degree of stretching results in most labeled daughter cells remaining on the basement membrane thereby reducing migration of labeled basal cells into the spinous layer.

The increased basal cell labeling accompanying slight to moderate stretching of the skin may result from several factors among which is absorption of the isotope through the expanded surface on the basement membrane. The subsequent increase in mitoses resulting in more basal cells would reduce the tension in that layer and restore the shape and intracellular tension of the basal cell population. This process may occur in normal growth. The reduction in labeling with further stretching may be because of the induced cell deformation and the collapse of the dermal vessels so that the radioisotope and nutrients cannot reach the cells. These processes may predispose to ulceration of skin under great tension as in leg edema or in areas of skin overlying abscesses.

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